

Phylogeny and Biogeography of *Paradoris* (Nudibranchia, Discodorididae), with the Description of a New Species from the Caribbean Sea

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Abstract. *Paradoris adamsae* sp. nov. is described based on three specimens collected in Bocas del Toro, on the Caribbean coast of Panama. The new species is clearly a member of *Paradoris* because of the presence of a jaw with three plates, a narrow radula, a grooved outer edge of the lateral tooth hook, and grooved oral tentacles. It differs from other members of the genus by having a relative large body size, brownish body color, small rhinophores, and high rounded tubercles on the mantle. This is the second record of the genus *Paradoris* in the western Atlantic, where *Paradoris mulciber* has been reported from Brazil, Costa Rica, and the Caribbean Sea. A phylogenetic analysis including 25 taxa shows that *Paradoris* is monophyletic and divided into two main clades, one containing all tropical eastern Pacific, tropical Atlantic, and Mediterranean species, which is sister to a clade composed of tropical and temperate Indo-Pacific species. In the Indo-Pacific clade, three taxa from the southern temperate seas are sisters to the rest of the Indo-Pacific species. According to the present phylogenetic hypothesis, *P. adamsae* sp. nov. is more close related to *Paradoris lopezi* Hermosillo & Valdés, 2004, from the tropical eastern Pacific than to *P. mulciber*, from the western Atlantic. The present phylogeny is similar in several regards to hypotheses proposed for other groups of opisthobranchs and other marine organisms and suggests that the same major vicariant events had affected the biogeography of these groups. As a result of the present study at least 16 distinct species can be recognized in *Paradoris*, more than the double the number of valid species cited in the last revision of the genus.

INTRODUCTION

The genus *Paradoris* is currently considered a member of the Discodorididae nudibranch clade (Valdés, 2002). *Paradoris* is characterized by having a jaw composed of a pair of lateral jaw plates with a third ventral plate, a narrow radula, grooved oral tentacles, and the radular tooth hook with a grooved outer edge. Accessory glands at the distal portion of the reproductive system, which are often associated with stylets, tubercles, and large holes on the mantle, are generally present in this group (Dayrat, 2006). Before a recent worldwide revision, 12 species names were considered as valid. Dayrat (2006) reexamined all available type material and examined newly collected specimens of described

species. Furthermore, this author transferred three additional species to *Paradoris* (*Discodoris erythraeensis* Vayssiére, 1912; *Discodoris lora* Marcus, 1965; and *Discodoris cavernae* Starmühlner, 1955), proposed new synonyms and identified three new morphotypes, which were called *Paradoris* sp. A, *Paradoris* sp. B, and *Paradoris* sp. C. The conclusion of Dayrat's revision is that *Paradoris* is composed by only eight valid species names and three unnamed taxa (see Dayrat, 2006:128,229). More recently Camacho-García & Gosliner (2007) described an additional species from South Africa. Furthermore, these authors noted that some specimens that Dayrat had suggested as conspecific, such as *Paradoris erythraeensis*, probably represent distinct species.

Paradoris is distributed throughout tropical and temperate oceanic areas, but most of the described

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species occur in shallow waters of the Indo-Pacific region. Two species are known from deep waters of the south Pacific, *Paradoris araneosa* Valdés, 2001, and *Paradoris imperfecta* Valdés, 2001. The present paper includes the description of a new species from the Caribbean Sea, which is the second record of the genus from the western Atlantic. The single species previously reported from this region was *Paradoris mulciber* (Ev. Marcus, 1970), described as *Percunas mulciber* based on specimens collected in northern Brazil.

In the present study we propose a new phylogenetic hypothesis for *Paradoris*. Two previous phylogenetic studies dealt with species of *Paradoris*. Dayrat & Gosliner (2005) included five species of *Paradoris* in a general phylogenetic analysis of the family Discodorididae and Dayrat (2006) conducted a phylogenetic analysis of *Paradoris* including 13 taxa, but he did not include all species, excluding for example *Paradoris lopezi* Hermosillo & Valdés, 2004. These two previous studies show the relations of *Paradoris* with other Discodorididae taxa but the phylogenetic relationships within *Paradoris* remained unresolved. The present study aims to provide a more comprehensive phylogenetic hypothesis for *Paradoris* species, including examination of patterns of biogeography.

MATERIAL AND METHODS

Taxonomy

Three specimens of a new species of *Paradoris* were collected in Bocas del Toro, on the Caribbean coast of Panama. All specimens were photographed alive to document color information. All material of the new species is deposited at the Natural History Museum of Los Angeles County (LACM). Two specimens were dissected through a dorsal incision. The internal features were examined and drawn using a stereomicroscope with *camera lucida*. Special attention was paid to the morphology of the reproductive system, including observations on the presence of accessory glands and stylet sacs. The buccal mass was removed and dissolved in 10% sodium hydroxide until the armed labial cuticle and the radula were isolated from the surrounding tissue. Then they were rinsed in water, dried, and mounted for scanning electron microscope (SEM) observation. Dorsal portions of the center and margin of the mantle were critical-point dried and mounted for SEM study. Other specimens from the California Academy of Sciences, San Francisco (CASIZ), and the South African Museum, Cape Town (SAM), are mentioned in the paper but have not been directly examined.

Phylogeny

The data were obtained from dissected specimens and from the literature (Table 1). In order to calculate the most parsimonious phylogenetic tree, data were analyzed by means of Phylogenetic Analysis Using Parsimony (PAUP) (version 4.0b4a, Sinauer Associates, Sunderland, Massachusetts) using the branch-and-bound algorithm. All characters were treated as unordered and unweighted. A Bremer support analysis (Bremer, 1994) was carried out to estimate branch support. In the cases in which the number of possible trees exceeded computer memory, the strict consensus was calculated using the first 10,000 trees obtained. Synapomorphies were obtained using the trace option in MacClade 4.08 (Sinauer Associates, Sunderland, Massachusetts) using the single most parsimonious tree from the PAUP analysis.

SYSTEMATICS

Family DISCODORIDIDAE Bergh, 1891

Genus *Paradoris* Bergh, 1884

Paradoris adamsae sp. nov.

(Figures 1–3)

Paradoris mulciber Ev. Marcus, 1970: Collin et al. 2005:692.

Paradoris sp. Valdés et al., 2006:180–181.

Type material

Holotype: Crawl Key, Bocas del Toro, Panama, 20 February 2004, 6 m depth, 1 specimen 45 mm preserved length (LACM 3094). **Paratypes:** Crawl Key, Bocas del Toro, Panama, 20 February 2004, 6 m depth, 2 specimens 45–50 mm preserved length, dissected (LACM 3095). SEM stubs with radula and labial cuticle deposited together the specimens. All specimens collected by A. Valdés.

Etymology: Dedicated to Peggy Adams and the Adams Foundation in gratitude for the internship in Biological Sciences at the Natural History Museum of Los Angeles County given to the senior author.

Geographic distribution: *Paradoris adamsae* sp. nov. is only known from the type locality, Bocas del Toro, along the Caribbean coast of Panama.

External morphology: Body oval and elevated. Dorsum covered with large, conical, irregular tubercles, some of them clearly larger than the rest. Larger and higher tubercles often situated on the dorsal hump. Small tubercles found on the mantle margin. Entire dorsum covered with small holes of different diameters (up to 100 µm). Short, perfoliate rhinophores composed of about 16 lamellae. Gill composed of six tripinnate

Table 1
Species included in the analysis, with the sources of information.

Taxa	Source of information
<i>Peltodoris atromaculata</i> Bergh, 1880	Valdés, 2002
<i>Peltodoris nobilis</i> (MacFarland, 1905)	Valdés, 2002
<i>Geitodoris planata</i> (Alder & Hancock, 1846)	Valdés, 2002
<i>Paradoris adamsae</i> sp. nov.	LACM 3094, LACM 3095
<i>Paradoris araneosa</i> Valdés, 2001	Valdés, 2001
<i>Paradoris caerulea</i> Camacho-García & Gosliner, 2007	Camacho-García & Gosliner, 2007
<i>Paradoris ceneris</i> Ortea, 1995	Ortea, 1995
<i>Paradoris dubia</i> (Bergh, 1904)	Bergh, 1904; Dayrat, 2006*
<i>Paradoris erythraeensis</i>	Dayrat, 2006*
<i>Paradoris imperfecta</i> Valdés, 2001	Valdés, 2001
<i>Paradoris indecora</i> (Bergh, 1881)	Bergh, 1881; Valdés, 2002
<i>Paradoris inversa</i> Ortea, 1995	Ortea, 1995
<i>Paradoris leuca</i> Miller, 1995	Miller, 1995; Dayrat, 2006*
<i>Paradoris liturata</i> (Bergh, 1905)	Dayrat, 2006
<i>Paradoris lopezi</i> Hermosillo & Valdés, 2004	Hermosillo & Valdés, 2004
<i>Paradoris lora</i> (Marcus, 1965)	Marcus, 1965
<i>Paradoris mollis</i> Ortea, 1995	Ortea, 1995
<i>Paradoris mulciber</i> (Marcus, 1970)	Marcus, 1970; Marcus, 1976; LACM 173261
<i>Paradoris tsurugensis</i> Baba, 1986	Baba, 1986; Dayrat, 2006
<i>Paradoris</i> sp. 1	Dayrat, 2006 (CASIZ 157029)
<i>Paradoris</i> sp. 2	Gosliner, 1987; Dayrat, 2006 (SAM A32370, SAM A35586)
<i>Paradoris</i> sp. 3	Dayrat, 2006 (CASIZ 099390)
<i>Paradoris</i> sp. 4	Dayrat, 2006 (CASIZ 089053, CASIZ 099080, CASIZ 105261, CASIZ 115827)
<i>Paradoris</i> sp. 5	Dayrat, 2006 (CASIZ 167456, CASIZ 110387)
<i>Paradoris</i> sp. 6	Dayrat, 2006 (CASIZ 072185)

* In these cases we only considered the remarks on the holotype and paratype (or paratypes).

branchial leaves, with four leaves pointing anteriorly and two leaves that border the anus pointing posteriorly. Ventrally, oral tentacles short, conical and grooved longitudinally. Anterior border of the foot grooved and notched. Dorsal color of living animal brown, with many small white cream spots and some dark dots covering the notum. Pale brown rhinophores with dark spots.

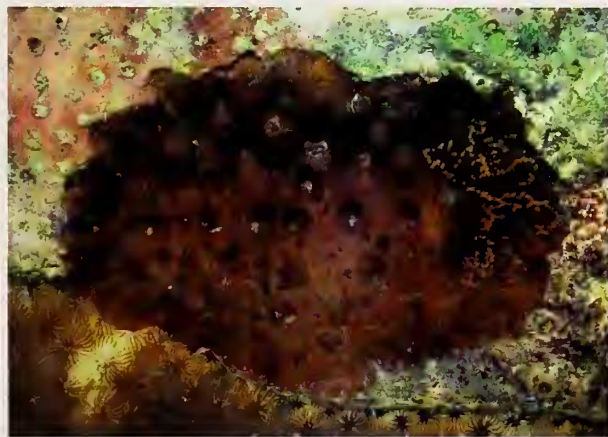


Figure 1. Dorsal view of the living holotype of *Paradoris adamsae* sp. nov. from Bocas del Toro, Panama (LACM 3094).

Grayish-brown branchial leaves scattered with dark spots. Tips of the branchial leaves orange/cream. Ventral color pale cream with few small brown dots.

Radula and jaw: Radular formula $65 \times 24.0.24$ in the 50 mm-long paratype and $68 \times 24.0.24$ in the 45 mm-long paratype (LACM 3095). Rachidian teeth absent. Lateral teeth hook-shaped with a grooved outer edge. Lateral teeth devoid of denticles. Innermost lateral teeth with a wide, large base and a thin, long cusp (Figure 2A). After the first three or four lateral teeth the shape changes to teeth with a stronger cusp, small spur, and short base (Figure 2C). Teeth increase in size from the innermost end to two-thirds of the half-row, where they began to decrease in size. Short outermost teeth; different in shape from all others; they can be a simple plate or have two irregular short cusps (Figure 2B). Labial cuticle armed with two lateral plates and one ventral plate. Each plate possesses numerous single, thin, and unicuspid rodlets with noncurved tips (Figure 2D).

Reproductive system: Reproductive system triaulic. Ampulla long; simple or with a single loop; it branches into a short oviduct and the prostate (Figure 3A). The oviduct enters the female glands mass in a depression near its

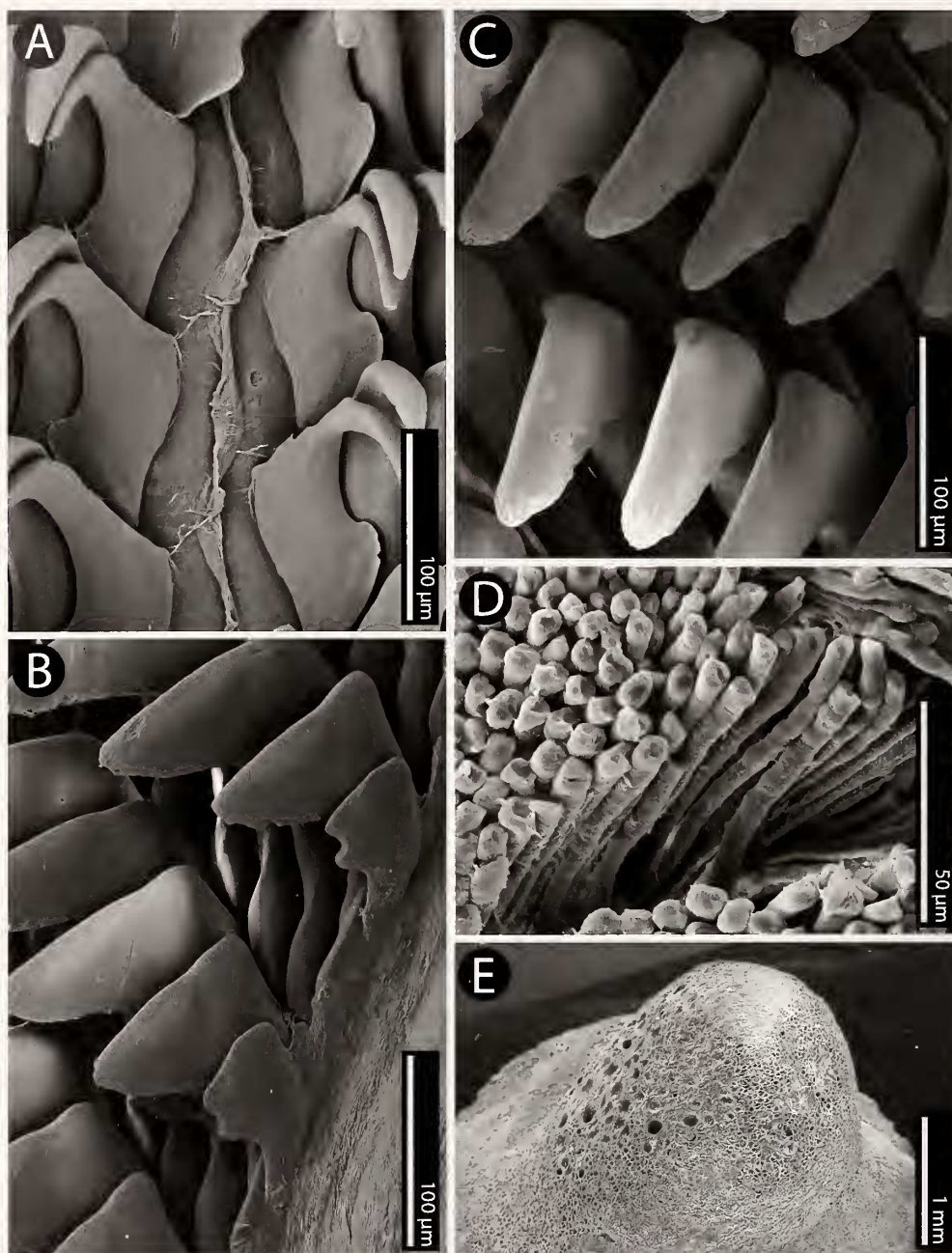


Figure 2. SEMs of a paratype of *P. adamsae* sp. nov. (LACM 3095). **A.** Innermost lateral radular teeth. **B.** Outermost lateral teeth. **C.** Midlateral radular teeth. **D.** Jaw rodlets. **E.** Dorsal tubercle showing the dorsal holes.

center. Prostate long and granular, divided into two portions clearly distinguishable by their different texture and color. Deferent duct with few, about three, loops; it opens into a common atrium with the vagina. Penis unarmed. The two paratypes dissected (LACM 3095) each have two accessory glands connected to the atrium (Figure 3B). Stylets sacs and stylets absent. Vagina long and thin, connects to a large, smooth and oval bursa

copulatrix. From the bursa copulatrix leads another duct that connects to an oval and muscular seminal receptacle and to a thin uterine duct. Bursa copulatrix about four times larger than the seminal receptacle.

Remarks: *Paradoris adamsae* sp. nov. is here regarded as a member of *Paradoris* because of the presence of grooved oral tentacles, an armed labial cuticle with a pair of lateral plates with a third ventral jaw plate, a

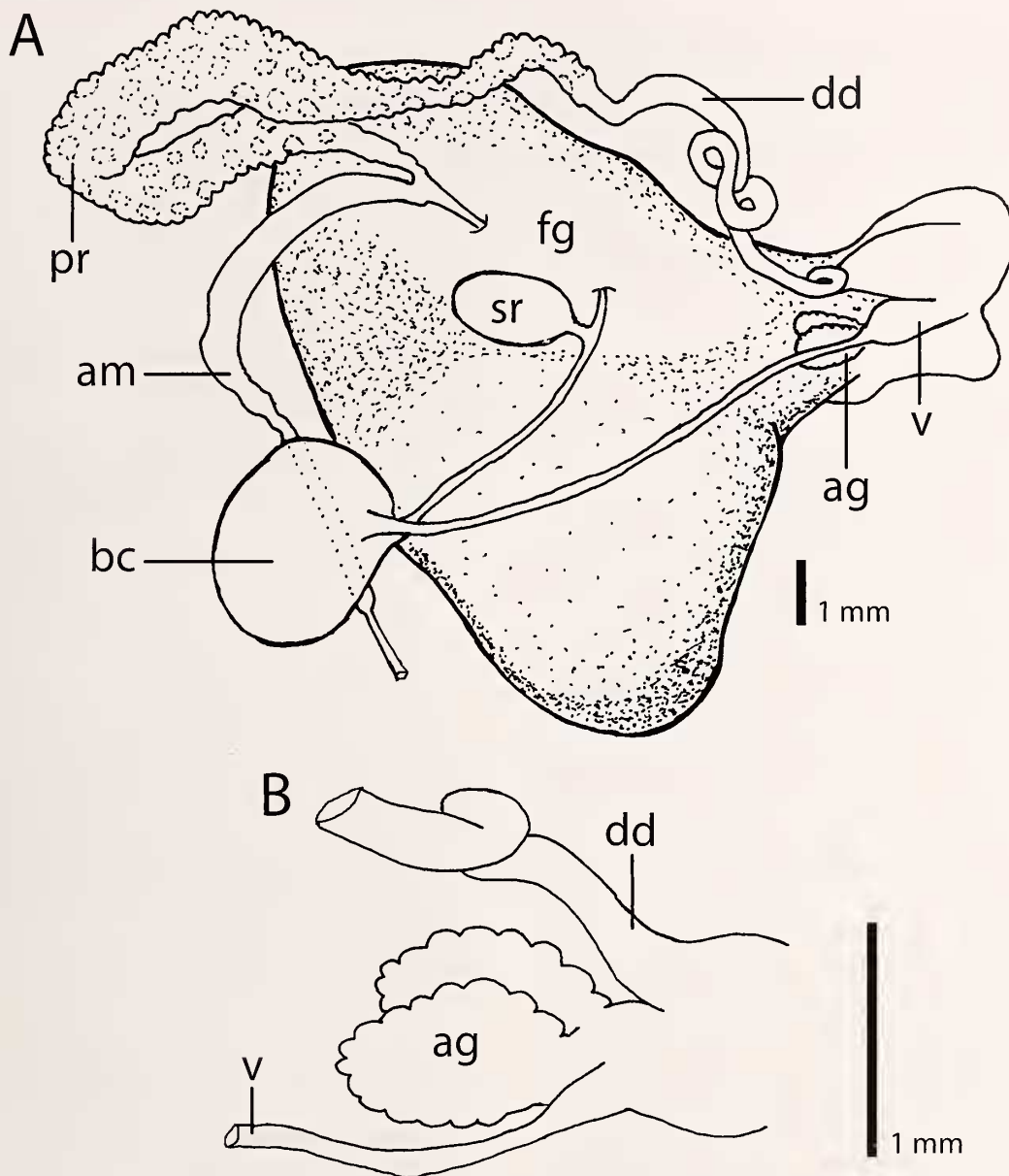


Figure 3. Reproductive system of a paratype of *P. adamsae* sp. nov. (LACM 3095). **A.** General view of the reproductive organs. **B.** Detail of the genital atrium showing the accessory glands. Abbreviations: ag, accessory gland; am, ampulla; bc, bursa copulatrix; dd, deferent duct; fg, female glands mass; pr, prostate; sr, seminal receptacle; v, vagina.

narrow radula, and a grooved outer edge of the lateral hook-shaped teeth, all characteristics of *Paradoris* (Dayrat, 2006).

Paradoris adamsae sp. nov. is different from other species of *Paradoris* described to date. *Paradoris lopezi* is similar to *Paradoris adamsae* sp. nov. because of the presence of large and conical tubercles on the dorsum, but these two species are easily distinguishable by their external coloration; *P. lopezi* is a pale grey species with

orange tubercle tips, whereas *P. adamsae* sp. nov. is dark brown with the tubercles having the same color as the rest of the dorsum. Anatomically, *P. lopezi* has stylet sacs with stylets, which are absent in *P. adamsae* sp. nov. The only other species described from the western Atlantic, *P. mulciber*, has been recently redescribed by Camacho-García & Gosliner (2007) and it is also distinguishable from *P. adamsae* sp. nov. in several regards. Externally, the tubercles of *P.*

mulciber are more rounded, less conical and more spaced than those of *P. adamsae* sp. nov., and the color of *P. mulciber* is paler than that of *P. adamsae* sp. nov. In the reproductive system, the main difference between these two species is the presence of stylet sacs with stylets in *P. mulciber*, which are absent in *P. adamsae* sp. nov.

PHYLOGENETIC ANALYSIS

Taxa

For the phylogenetic analysis, 25 taxa have been considered. All nominal species of *Paradoris*, including species recently synonymized by Dayrat (2006) were included, in addition to *Paradoris adamsae* sp. nov. Representatives of other Discodorididae taxa have been included in the analysis for outgroup comparative purposes, these include *Peltodoris atromaculata* Bergh, 1880, *Peltodoris nobilis* (MacFarland, 1905), and *Geitodoris planata* (Alder & Hancock, 1846). Data on species were obtained from various sources (Table 1).

As Camacho-García & Gosliner (2007) observed, the specimens referred as *P. erythraeensis* by Dayrat (2006) constitute a group of species easily distinguishable by internal and external characteristics. For the present analysis, the data for *P. erythraeensis* were obtained from the description of the type material of this species. From the group of specimens considered as *P. erythraeensis* by Dayrat (2006), three different species were identified and are here called *Paradoris* sp.1 (CASIZ 157029), *Paradoris* sp. 2 (SAM A32370, SAM A35586), and *Paradoris* sp. 3 (CASIZ 099390). Dayrat's (2006) species *Paradoris* sp. B and *Paradoris* sp. C are here referred as *Paradoris* sp. 4 and *Paradoris* sp. 5, respectively. Specimen CASIZ 089053 was excluded from other specimens that belong to *Paradoris* sp. 4. This specimen differs from other members of Dayrat's *Paradoris* sp. B by having tubercles on the dorsal hump and may constitute a different species. It is not included in the present phylogenetic analysis because it is an immature specimen and no reproductive system was found (Dayrat, 2006).

Dayrat's *Paradoris* sp. A is also not included at the analysis. The description of *Paradoris* sp. A was made based on 11 specimens collected in four different localities and, as in the case of *P. erythraeensis*, these specimens present a wide range of morphological and anatomical differences and appears to correspond to a group of species. Because there were no morphological data available of most of these specimens, we prefer to not include *Paradoris* sp. A in the present phylogenetic analysis. The specimen CASIZ 072185 studied by Dayrat (2006) is included in the analysis and herein called *Paradoris* sp. 6.

Characters

The characters used to resolve the phylogeny of *Paradoris* are detailed below. They reflect a range of morphological and anatomical features of the taxa involved. The original list of characters was in part based on the characters selected by Dayrat (2006) and subsequently modified to include additional characters that appear to be more informative. Eleven characters are coded as binary and five characters as multistate. Character states are indicated with numbers: 0, plesiomorphic condition; 1–4, apomorphic conditions. The polarities discussed below have been obtained as the result of outgroup comparison. A data matrix of character states is found in Table 2.

1. **Dorsal brownish-black patches:** Present in *Peltodoris atromaculata*, *Peltodoris nobilis*, and 10 species of *Paradoris* (1). Absent in all other taxa studied (0). In species with dark background color this character is treated as not applicable.
2. **Dorsal brownish-black patches pattern:** Four states are recognized for this character. Some species including *Paradoris lenca* Miller, 1995 and *Paradoris* sp. 1 have irregular dark dots (0). *Paradoris* sp. 4 and *Paradoris* sp. 5 have short stripes (1). In *P. liturata* the stripes are long, making a network at the dorsum (2). *Peltodoris atromaculata* has large and ovoid patches (3) and *Paradoris caerulea* Camacho-García & Gosliner, 2007 has two circular patches at the dorsal hump (4). In species with dark background color or species without dorsal dark patches this character is treated as not applicable.
3. **Gill apex color:** In *P. araneosa* and *P. lora* (Marcus, 1965) the gill apex color is the same as the rest of the gill (0). In *Paradoris indecora* (Bergh, 1881) and *P. lopezi* the gill apices have a distinct color (1). The character state could not be determined for *Paradoris mollis* Ortea, 1995 and *Paradoris* sp. 1.
4. **Oral tentacles:** They are grooved in all *Paradoris* species (1). In the outgroup taxa this condition is absent (0) and it is considered the plesiomorphic state.
5. **Mantle:** Most species of Discodorididae have dorsal structures such as tubercles and/or granules (0). The mantle is smooth in *Paradoris* sp. 4 and *Paradoris* sp. 5, which is considered the apomorphic state (1). Specimens of *Paradoris dubia* (Bergh, 1904) may present dorsal tubercles or not.
6. **Dorsal tubercle size and arrangement:** *Peltodoris atromaculata*, *Paradoris mulciber*, *P. lopezi* and *P. adamsae* sp. nov. have large tubercles on the central part of dorsum (0). *Paradoris indecora* and *Paradoris ceneris* Ortea, 1995 have large tubercles on the margin of the dorsum (1). Similar-sized or

Table 2

Data matrix of character states used in the phylogenetic analysis. The character states are indicated with numbers: 0, plesiomorphic condition; 1–4: apomorphic conditions. Question marks indicate unknown data. Dashes indicate nonapplicable characters.

Species	Character state																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
<i>P. atomaculata</i>	1	3	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
<i>P. nobilis</i>	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>G. planata</i>	0	–	1	0	0	2	0	0	1	0	0	0	0	0	0	0	0	1	0
<i>P. adamsae</i> sp. nov.	0	–	1	1	0	0	1	1	2	1	0	1	0	0	0	0	0	1	0
<i>P. araneosa</i>	0	–	0	1	0	2	0	0	2	1	0	1	0	0	0	0	0	1	1
<i>P. caerulea</i>	1	4	0	1	0	2	1	0	2	1	0	1	0	1	0	0	0	1	1
<i>P. ceneris</i>	0	–	1	1	0	1	1	0	2	1	0	1	1	0	0	1	0	0	0
<i>P. dubia</i>	1	0	0	1	0/1	2	0/1	0	2	1	0	1	0/1	0	1	0	0	0	0
<i>P. erythraeensis</i>	1	0	?	1	0	?	0	0	2	1	0	1	1	?	0	0	0	1	1
<i>P. imperfecta</i>	0	–	0	1	0	2	0	0	2	1	0	1	0	1	0	0	1	0	1
<i>P. indecora</i>	0	–	1	1	0	1	1	0	2	1	0	1	1	0	0	0	0	1	1
<i>P. inversa</i>	0	–	1	1	0	1	1	0	2	1	0	1	1	0	0	0	0	1	1
<i>P. leuca</i>	1	0	0	1	0	2	0	0	2	1	0	1	1	0	1	1	0	0	0
<i>P. liturata</i>	1	2	0	1	0	2	0	0	2	1	1	1	1	0	0	0	0	1	1
<i>P. lopezi</i>	0	–	1	1	0	0	1	1	2	1	0	1	1	0	0	0	0	1	1
<i>P. lora</i>	0	–	0	1	0	2	0	0	2	1	0	1	0	1	0	0	0	?	?
<i>P. mollis</i>	0	–	?	1	0	1	1	0	2	1	0	1	1	1	0	0	0	1	1
<i>P. mulciber</i>	0	–	1	1	0	0	1	0	2	1	0	1	1	0	0	0	0	1	1
<i>P. tsurugensis</i>	1	0	0	1	0	2	1	0	2	1	0	1	0	0	?	0	0	1	1
<i>Paradoris</i> sp. 1	1	0	0	1	0	2	0	0	2	1	0	1	1	0	0	1	0	1	1
<i>Paradoris</i> sp. 2	1	0	0	1	0	2	0	0	2	1	0	1	?	0	0	0	0	0	0
<i>Paradoris</i> sp. 3	0	–	0	1	0	2	0	0	2	1	1	1	0	0	0	1	0	1	1
<i>Paradoris</i> sp. 4	1	1	1	1	1	–	0	0	2	1	1	1	0	0	0	0	0	0/1	0/1
<i>Paradoris</i> sp. 5	1	1	1	1	1	–	0	0	2	1	0	1	?	0	0	?	?	1	1
<i>Paradoris</i> sp. 6	0	–	0	1	0	2	0	0	2	1	1	1	0/1	0	?	?	0	1	1

large tubercles distributed all over the dorsum, which occur in *P. lora* and *P. imperfecta*, represent another apomorphic state (2). The character state could not be determined for *Paradoris* sp. 1.

7. **Dorsal large granular tubercles:** In *P. lopezi* and *P. mulciber* there are some large granular tubercles on the dorsum (1). *Paradoris liturata* Bergh, 1905 has large tubercles but they are not granular and *P. erythraeensis* has no large tubercles (0).
8. **Dorsal high granular tubercles:** *Paradoris adamsae* sp. nov. and *P. lopezi* have high granular tubercles at the dorsum (1). In *P. liturata* there are some high tubercles but they are not granulated and all other *Paradoris* taxa lack highly elevated tubercles (0).
9. **Jaw:** *Peltodoris nobilis* has a smooth labial cuticle with no rodlets (0). *Geitodoris planata* has a labial cuticle armed with a pair of lateral jaw plates (1). All *Paradoris* species have the labial cuticle armed with a pair of lateral jaw plates and a ventral plate (2).
10. **Radula width:** All *Paradoris* species have a narrow radula relative to length (1). The absence of this condition is considered the plesiomorphic state and it is present in all members of the outgroup (0).

11. **Radula symmetry:** Some *Paradoris* species have an asymmetrical radula with a higher number of teeth on the left side than the right side (1). All other known Discodorididae have symmetrical radulae (0).
12. **Outer edge of lateral teeth hook:** All *Paradoris* species have a grooved outer edge of the lateral tooth hook (1). The absence of a groove in the outer edge of the tooth hook is considered plesiomorphic (0).
13. **Outermost tooth size:** In *P. lopezi* and *P. mulciber* the outermost teeth are very reduced compared to the lateral teeth (1), in *P. araneosa* and *P. imperfecta* the outermost tooth are of similar size to the adjacent lateral teeth (0). The asymmetrical radula of *Paradoris* sp. 6 has outermost teeth with different sizes on each side of the radula (0/1). The character state could not be determined for *Paradoris* sp. 2 and *Paradoris* sp. 5.
14. **Ampulla length:** *Paradoris imperfecta*, *P. mollis*, and *P. lora* all have a short ampulla (1). All other species have a long ampulla (0). The character state could not be determined for *Paradoris* sp. 1.
15. **Prostate shape:** The majority of Discodorididae

- have a flattened prostate (0). Some *Paradoris* species have a tubular prostate (1).
16. **Relative size of bursa copulatrix/seminal receptacle:** A larger bursa copulatrix is considered the plesiomorphic state (0). *Paradoris leuca* and *P. ceneris* have a bursa copulatrix with similar size to that of the seminal receptacle (1).
 17. **Seminal receptacle surface:** In *Peltodoris atrouaculata* and *Paradoris imperfecta* the seminal receptacle surface is irregular (1). All other species studied have smooth seminal receptacle surface (0).
 18. **Accessory glands:** Some Discodorididae have accessory glands at the distal portion of the reproductive system. The presence of these glands is considered the apomorphic state of this character (1). The majority of *Paradoris* species have accessory glands but *P. dubia* and *P. ceneris* lack these structures (0). According to Dayrat (2006), specimens of *Paradoris* sp. 4 may or may not present accessory glands. The character state could not be determined for *P. lora*.
 19. **Stylet sacs and stylets:** Some Discodorididae have stylet sacs with stylets at the distal portion of the reproductive system. The majority of *Paradoris* species have these structures and this is the apomorphic state of the character (1). *Peltodoris atrouaculata*, *P. uobilis*, and *Paradoris dubia* lack these structures (0). According to Dayrat (2006) specimens of *Paradoris* sp. 4 may present or not stylet sacs. The character state could not be determined for *P. lora*.

PHYLOGENETIC RESULTS

Phylogenetic analysis of the data matrix resulted in a single most parsimonious tree, with a length of 46 steps, a consistency index of 0.522 and a retention index of 0.722. The tree is shown in Figure 4, including character numbers to trace character evolution. Bold and italic numbers indicate reversals and larger numbers on the lower side of the branches indicate Bremer support analysis results.

The single tree shows that *Paradoris* is a monophyletic group supported by five synapomorphies: gill apex color (3), grooved oral tentacles (4), presence of an armed labial cuticle with a pair of lateral plates and a third ventral jaw plate (9), narrow radula (10), and lateral teeth hook with a grooved outer edge (12). Within *Paradoris*, there are two major clades. One contains all known tropical and subtropical eastern Pacific, Atlantic, and Mediterranean species. *Paradoris ceneris* is the most basal member of this clade. There is a polytomy including *P. indecora*, *P. mollis*, and *P. inversa* Ortea, 1995 (eastern Atlantic taxa), which are sister taxa to a clade containing western Atlantic and eastern Pacific species. *Paradoris adamsae* sp. nov. from the

Caribbean of Panama appears to be more closely related to *P. lopezi* (from the eastern Pacific) than to *P. mulciber* (from Brazil and the Caribbean Sea).

The second main clade includes a monophyletic assemblage of temperate and tropical Indo-Pacific species distributed in two sister groups. One is composed of three temperate taxa: *P. dubia*, *P. leuca*, and *Paradoris* sp. 2, from southern temperate waters of Australia, New Zealand, and South Africa, respectively. The other clade constituted by the remaining Indo-Pacific species, including other two temperate species, *P. caerulea* from South Africa and *Paradoris tsurugensis* Baba, 1986 from Japan that are sister, but derived species. In this second main clade there seems to be a pattern of monophyly related to geographic range, with species clades or species pairs arranged in latitudinal and longitudinal layers around the central Pacific and Indian oceans, providing a biogeographic pattern, possibly related to vicariant events at the edge of the central Pacific Ocean (Figure 5).

DISCUSSION

Tree Resolution and Characters

The present phylogenetic tree is highly resolved in comparison to previous phylogenetic hypothesis proposed by Dayrat (2006). The removal of continuous/noninformative characters used by Dayrat (2006), as relative to the jaw rodlets and the angle of the radular rows; the selection of additional informative characters; and the inclusion of many morpho-species that Dayrat (2006) synonymized into the same taxa probably account for the different results.

Phylogeny and Biogeography

The topology of the phylogenetic tree of *Paradoris* here presented, in which tropical eastern Pacific and Atlantic species constitute a monophyletic group, is similar to hypotheses proposed for other groups of nudibranchs (Gosliner & Johnson, 1999; Garovoy et al., 2001; Dorgan et al., 2002; Alejandrino & Valdés, 2006), other marine invertebrates such as sea urchins (Lessios et al., 1999; McCartney et al., 2000), shrimps (Baldwin et al., 1998), and also vertebrates such as reef fishes (Rocha, 2003). In most of these cases there is a repeated pattern in which the monophyletic group composed by tropical eastern Pacific and Atlantic species is sister to another monophyletic group containing tropical Indo-Pacific species. It has been proposed that the origin of this biogeographic pattern is related to two consecutive vicariant events: (1) the closure of the Tethys Sea approximately 20 million years ago (Mya), which communicated the Indo-Pacific region with the Atlantic and eastern Pacific and (2) the rise of the Panama isthmus approximately 3.1 Mya (see

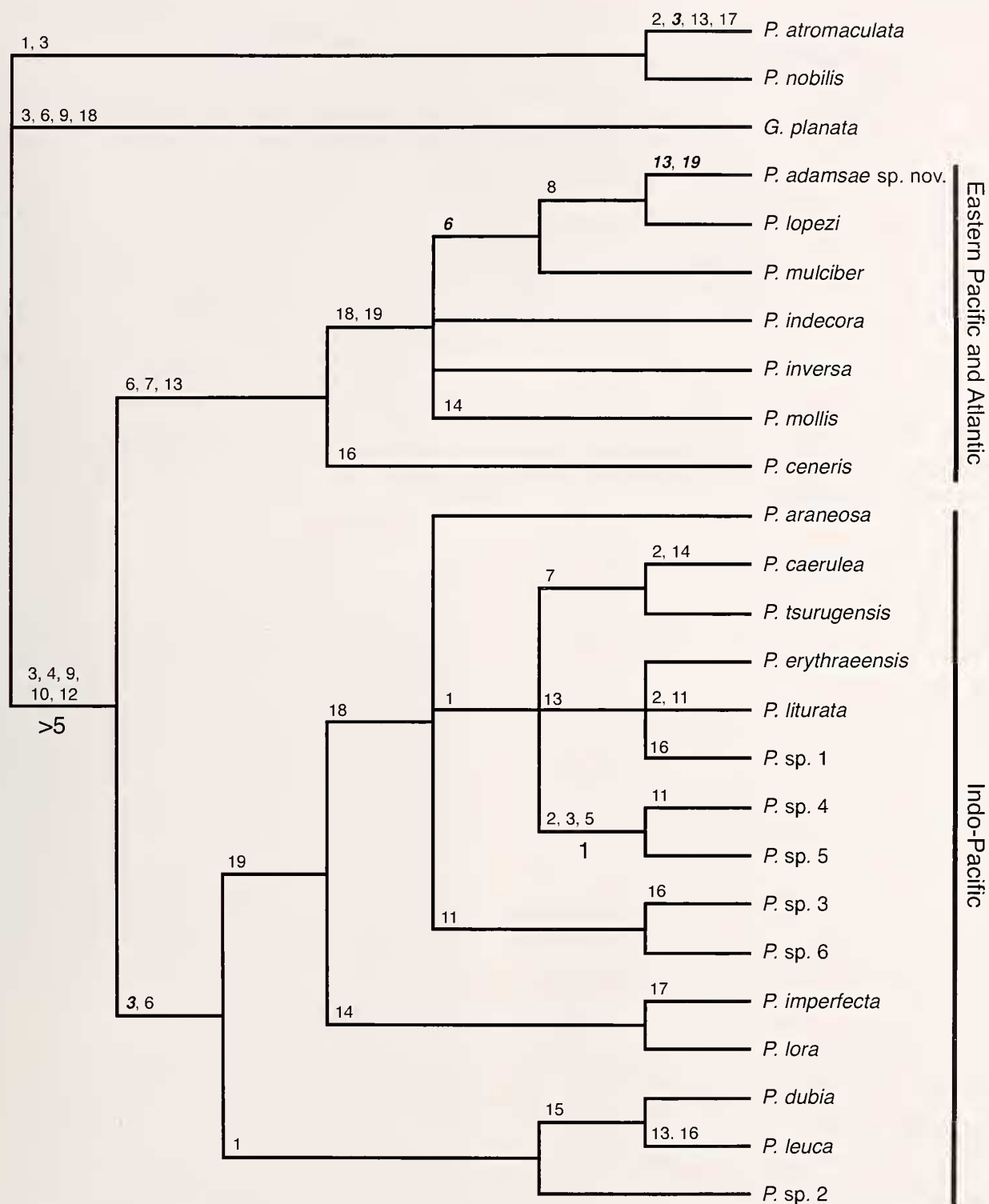


Figure 4. Phylogenetic hypothesis of *Paradoris*. Numbers above the branches show character evolution. Numbers in bold italics show cases of reversal and parallel evolution. Larger numbers below the branches show the results of the Bremer support analysis.

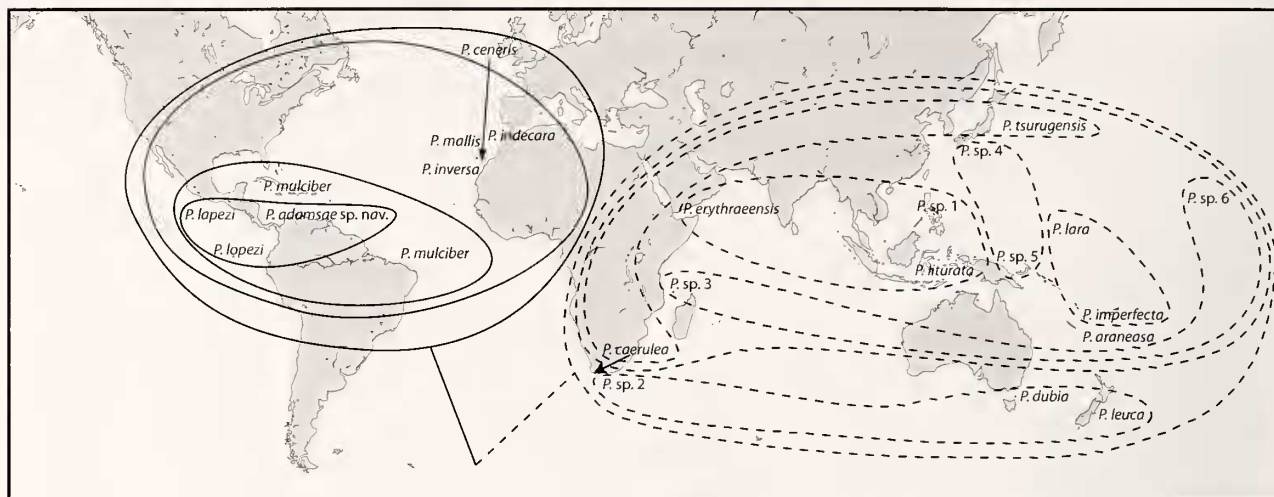


Figure 5. Map showing the biogeographic signal provided by the phylogenetic hypothesis. Species names are situated on the map in the approximate areas where the species occurs; due to space limitations, an arrow points to the actual geographic range of *P. caerulea*. Taxa and clades with sister relationships in the phylogenetic hypothesis are encircled together. Line patterns indicate to which of the main two clades of *Paradoris* each taxon or clade belongs; for a description of the main two clades see the Results section.

Coates & Obando, 1996; Badlwin et al., 1998; Lessios, 1999; McCartney et al., 2000; Valdés, 2004). It seems that these events, and the fact the eastern Pacific is isolated from the western Pacific by the East Pacific Barrier, would produce effective isolation that allowed allopatric speciation.

In the present phylogenetic hypothesis three southern temperate species (*Paradoris dubia*, *P. leuca*, and *Paradoris* sp. 2) are grouped in a sister clade to the remaining Indo-Pacific *Paradoris* (Figures 4, 5), which are mostly tropical species. A similar pattern can be observed in other dorid nudibranchs such as *Rostanga* (Garovoy et al., 2001) and *Acanthodoris* (Fahey & Valdés, 2005). In the phylogenetic hypothesis of *Rostanga*, a small clade containing South African and subarctic species is sister to a clade with all other species (Garovoy et al., 2002). The phylogenetic hypothesis proposed for *Acanthodoris* shows that the basal species of this group are found in temperate southern waters of South America, South Africa, and Australia (Fahey & Valdés, 2005). This recurrent pattern suggests either a southern temperate origin of these groups or an early divergence of southern temperate and tropical taxa. In the case of *Paradoris*, other temperate species such as *P. tsurugensis* (from Japan) and *P. caerulea* (from South Africa) are derived members of the Indo-Pacific clade, which seem to suggest that these species derived from tropical Indo-Pacific ancestors.

Taxonomic Remarks

Dayrat (2006) recognized only eight valid species names in *Paradoris*: *P. araneosa*, *P. dubia*, *P. ery-*

thraeensis, *P. indecora*, *P. liturata*, *P. lopezi* and *P. tsurugensis*. He also proposed six new synonyms for *Paradoris* species. Camacho-García & Gosliner (2007) criticized the taxonomy proposed by Dayrat (2006) on the basis that in some cases Dayrat defined species based on the presence of a shared characteristic between several specimens, rather than on the correlation of characters, which appears to produce better estimations of species boundaries and diagnostic characters. In light of Camacho-García & Gosliner's (2007) paper, it becomes clear that the synonymies proposed by Dayrat (2006) require a careful reevaluation.

Dayrat (2006) may be correct about the synonym of *P. dubia* and *P. leuca*, species described from southern Australia and New Zealand, respectively. We were unable to detect any consistent anatomical difference between these two species with the exception of the presence of a bursa copulatrix with similar size to that of the seminal receptacle in *P. leuca*. However, some differences in the dorsal color appears to separate these species (Rudman, 2007).

The synonymy proposed by Dayrat (2006) for *P. inversa* and *P. mollis*, species described from the Canary Islands by Ortea (1995), with *P. indecora*—known from the Canary Islands, Morocco, the Atlantic coast of Portugal, and the Mediterranean—also seems to be based on a correct interpretation of data; however, the lack of mention of accessory glands and stylet sacs in the original description of the single dissected specimen of *P. ceneris*, also synonymized with *P. indecora*, may indicate that this is indeed a distinct species from *P. indecora*. Finally, we disagree with Dayrat's (2006) proposal to synonymize *P. araneosa*

and *P. imperfecta*, two species from deep waters off south New Caledonia. Differences between these two species include the size of the ampulla, the morphology of the seminal receptacle surface, and the outermost tooth size. They are clearly two different species that according to the phylogenetic hypothesis here proposed do not share an immediate common ancestor. Furthermore, the documented external morphology and coloration of living animals of these two species is dramatically different (see Valdés, 2001).

The specimen here referred as *Paradoris* sp. 1 (CASIZ 157029) may belong to *P. erythraeensis* but the absence of data in the description of this specimen and differences found by Dayrat (2006) and here observed in the relative size of the bursa copulatrix and the seminal receptacle do not allow a definitive conclusion. As a result of the present study we recognize at least 16 distinct *Paradoris* species: *P. adamsae* sp. nov., *P. araneosa*, *P. caerulea*, *P. dubia*, *P. erythraeensis*, *P. imperfecta*, *P. indecora*, *P. liturata*, *P. lopezi*, *P. mulciber*, *P. tsurugensis*, *Paradoris* sp. 2, *Paradoris* sp. 3, *Paradoris* sp. 4, *Paradoris* sp. 5, and *Paradoris* sp. 6. This is more than the double of valid species cited in the last revision of the genus (Dayrat, 2006).

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